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IDENTIFICATION OF RAT MEATBALLS IN TRADITIONAL MARKET IN AREA OF JAKARTA USING REAL TIME–PCR

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Abstrak

Daging merupakan salah satu sumber protein yang banyak dikonsumsi. Mengkonsumsi makanan halal adalah kewajiban setiap muslim. Daging sapi dan daging ayam merupakan makanan yang paling banyak dikonsumsi di Indonesia. Daging olahan dengan bahan dasar sapi dan ayam yang paling banyak dikonsumsi menjadi bakso. Biasanya makanan olahan daging dari sapi sering dipalsukan dengan daging tikus. Tikus umumnya menjadi hama di Indonesia, seperti di persawahan dan gudang penyimpanan padi, Tikus memiliki bulu berwarna jingga kecokelatan dan berukuran antara 304-400mm. Rattus atau tikus hitam adalah tikus yang dapat ditemukan di seluruh dunia. Teknik analisis biologi molekuler RT-PCR tersebut menjadi teknik yang menjanjikan untuk mendeteksi atau mengidentifikasi DNA kontaminan dalam pangan olahan daging. Semua sampel hasil ekstraksi kemudian dianalisis kemurniannya dan memiliki kisaran kemurnian antara 1,8 hingga 1,89. Hasil Real Time PCR untuk deteksi kontaminan DNA tikus pada sampel bakso menunjukkan bahwa semua sampel (empat puluh enam sampel) yang dianalisis, lima sampel menunjukkan sampel terdeteksi negatif dan empat puluh satu sampel memiliki rentang nilai Cp antara 27,02 hingga 37,08. Terindikasi positif dari sampel hasil amplifikasi, namun nilai Cp yang terindikasi positif cenderung jauh lebih tinggi dibandingkan dengan nilai Cp kontrol positif yaitu 16,26.

Kata kunci: DNA, Bakso, Tikus, RT-PCR

Abstract

Identification of Rat Meatballs In Traditional Market In Area of Jakarta Using Real Time–PCR. Meat is one of protein source who was often consumed. Consume halal food are obligation for every moslem. Beef and chicken meat is the most popular food consumed in Indonesia, processed beef and chicken meat is the most consumed, example meatballs. Usually processed meat food from beef are often counterfeiting with rat meat. Rat is generally become pests in Indonesia, such in rice fields and rice storage warehouses, Rat has orange-brown fur and has sized between 304-400 mm. Rattus rattus or black rats are mice that can be found all over the world. The analysis technique in biology molecular, such RT – PCR becomes a promises technique for detection or identification contaminant DNA in meat processed food. All of the samples has been result of extraction then analyzed the purity and has purity range between 1.8 to 1.89. Results of Real Time PCR to detection rat DNA contaminants in meatball samples showed that all of the samples (forty-six samples) who was analyzed, five samples showed negative detected samples and forty-one other samples has Cp value range between 27.02 to 37.08, were indicated to be positively indicated from amplified samples, but the Cp value indicated in the samples was indicated positive tend to be much higher than the positive control Cp value, which is 16,26.

Keywords*: DNA, Meatballs, Rat, RT-PC*

INTRODUCTION

Meat can be processed by direct cooking or being processed food. Meat consumption needs of the Indonesian population increase in line with the increasing population of Indonesia and public awareness of the importance of animal protein. Consumption of animal protein certainly does not have to pay attention to quality, especially the safety of food source. The often problem of meatballs is the substitution or mixing of beef and chicken with meat that can carry diseases such as Rat meat.

Rat in Indonesia are divided into three type, Rattus argentiventer often become a are mice that generally become pests in rice fields and rice storage warehouses, have orange-brown fur and has sized between 304-400 mm. Rattus rattus or black rats are mice that can be found all over the world, which often can be found in our house and have yellow patterns on the ears. Rattus rattus who have switched to quieter places then adapt to the environment which is then referred to as Norwegian rats. Rattus norvegicus which has a body covered with skin and coarse brown hair and getting brighter in their lower body.

Food safety is the condition who was needed for prevent contamination from biology, chemical and other ingredients can be caused illness (PP RI 2004). The commission of Europe has been making rules of food safety that every part of food supply chain must to know every ingredients who was used in food process production. Food safety is defined as the conditions and efforts needed to prevent food from the possibility of biological, chemical and other material contamination that can disrupt, harm and endanger human health (PP RI, 2004). The European Commission has regulated food safety regulations that every part in the food supply chain must to know every raw material who was used in food production process (Ali 2012)

The existence of regulations and laws that regulate the safety of food products should be able to cause consumers a sense of security in consuming meat or processed products. However, there are still cases of counterfeit processed meat products found by mixing other animal meat in the process of making products to reduce the production costs, so the similar products can be obtained at a much lower cost.

One of technique who can be used for make sure food safety is species identification. Species identification is relevant for consument concern in economics, healthy, or religion. However, this method has deficiency such as can be used for species identification product who was through heating process, because protein tend degradated than DNA who was stable when heating process even when DNA fragmented, identification method using DNA still can be identification DNA from different species in sample (Farag 2015). PCR is the analysis technique in biology molecular for amplification one or some of copies from DNA in some sequences that resulting thousands to millions DNA sequences (Joshi 2011).

MATERIALS AND METHOD

A. Materials

Research material is meat processed product (meatballs) with a total of samples is forty five samples and purchased directly from street vendors in traditional markets without packaging and labels. Materials who was using for analysis is DNeasy Mericon Food, Chloroform, Mouse Positive Control and QuantiNova Sybr Green.

B. Metode

DNA Extraction

Prepare 200 mg samples and adding 1 ml food lysis buffer (can be directly 1 ml or gradually 500 µl and then 500 µl, depends on the samples itself). Extraction process continue with prepare 1 ml of samples and adding 2.5 proteinase – K, vortex and then incubation in temperature 60^0C, 30 minutes. Centrifuge with 2500xg, 5 minutes and added 500 µl Chloroform into new tube 2 ml. Moving clear layer from lysis tube, without touching precipitation in the tube. Added of 500 µl Chloroform, vortex for 15 seconds, and centrifuge with 14000xg, 15 minutes. Separate the clear supernatant and calculate the volume, then added 1:1 volume of buffer PB and vortex for 15 seconds. Place all liquids into Qiaquick spin column and centrifuge 17900xg for 1 minute. Dispose the liquid and reuse again the collection tube, added 500 µl of buffer AW2, centrifuge 17900xg for 1 minute, discard the supernatant. Place it Qiaquick spin column in new collection tube (2 ml) and centrifuge again 17900xg for 1 minute for dry the membrane. Dispose the collection tube and place the Qiaquick spin column in new collection tube (1.5 ml). Added 150 µl of buffer EB and incubation at room temperature for 1 minute, then centrifuge for 1 minute. DNA can be used directly for PCR or store in temperature of -20^0C.

Analysis Purity of DNA

Analysis can be done using instrument of Tecan Nanodrop Spectrophotometer. First, doing

blank with Elussion Buffer in plate, then continue with the DNA samples has been result of extraction. Samples 2 mikrolit and put it on the Tecan plate, reinsert the plate into the instrument, and then read the DNA purity using blank EB. DNA purity results will come out in the form of a table that includes the ratio and concentration of DNA.

RT–PCR Analysis

RT–PCR testing with instrument of Real Time–PCR Roche LC480. The testing doing by making master mix solution first, with following the rules of PCR Mix QuantiNova Sybr Green PCR Kit:

a. Dilute (2x) of QuantiNova Sybr Green PCR Mix, QuantiNova Yellow template dilution buffer,primer mix, RNAse Free water dan template DNA (if stored in –20°C);
b. Spin down for 15 seconds;
c. Making the PCR Mix into LifeTouch Microcentrifuge tube 1.7 ml, following the instruction below:

Component	X 1 (µl)	Final Concentration
2x QuantiNova Sybr Green PCR Master Mix	10 µl	1x
Primer Forward (10 µM)	1 µl	0,5 µM
Primer Reverse (10 µM)	1 µl	0,5 µM
Rnase Free Water	6 µl	
Template DNA **(added in step e)**	2 µl	≤100 ng/reaksi
Total volume reaksi	20 µl	

d. Mix the PCR fix with the amount of sample except template, then distributed into reaction tube 0.2 ml each 18 µl;
e. Added 2 µl template DNA (≤500 ng/reaksi) into PCR tube with PCR Mix.;
f. Set the RT – PCR machine as below:

Step	Time	Temperature
PCR initial activation enzim	2 min.	95°C
2-step cycling:		
Denaturation	5 s	95°C
Annealing	10 s	61°C*
Jumlah cycle : 35-40 cycle		
Melt: on Green 60 – 95		

RESULT AND DISCUSSION

The main focus of authentication for muslim consumers is on substitution of rats in meat processed. DNA molecules are targeted components for species identification rather than using protein, because the stability differences between the components and the amounts in biology tissue (Hamzah 2014). In this study, all of samples as a result of extraction analyze the purity of DNA using Tecan Nanophotometer with wavelength 260/280 nm for specific analysis of purity samples. Wavelength 260/280 nm is specific wavelength for analysis the DNA purity. The result of all samples has purity range of 1.8 – 1.89.

DNA samples that had reached the DNA purity requirements for testing using RT-PCR were then detected by DNA contaminants of rats using Real Time - PCR. In the detection of rat contaminants using Real Time - PCR, used Sybr Green, where the primers used will be denatured at annealing temperature during the PCR process. Results from Real Time PCR to detect rat DNA contaminants in meatball samples showed that all of the samples (forty-six samples) who was analyzed, five samples showed negative detected samples and forty-one other samples were indicated to be positively indicated from amplified samples, but the Cp value indicated in the samples was indicated positive tend to be much higher than the positive control Cp value, which is 16.26, while in this study Cp value was range between 27.02 to 37.08. This can be caused by samples that are positively indicated as amplified with Sybr Green but may not necessarily contain Rat DNA, on the other hand unamplified samples might be due to the low concentration of DNA in the sample.

Table 2. Result of Rat Contaminant Testing In Meat Processed

No	Area	Samples Code	Weight	DNA Concentration	Cp Value	Result
1	Jakarta Utara	SK1	261.4 mg	1,85	30.95	-
2	Jakarta Utara	SK2	234 mg	1,84	32.68	-
3	Jakarta Utara	SK3	266 mg	1,85	28.60	-
4	Jakarta Utara	KG1	269 mg	1,84	36.44	-

5	Jakarta Utara	KG2	270.3 mg	1,88	35.97	-
6	Jakarta Utara	KG3	244.4 mg	1,84	-	-
7	Jakarta Utara	KB1	249.1 mg	1,86	30.79	-
8	Jakarta Utara	KB2	229.8 mg	1,87	33.99	-
9	Jakarta Utara	KB3	244 mg	1,83	-	-
10	Jakarta Selatan	BD1	249.5 mg	1,84	31.15	-
11	Jakarta Selatan	BD2	242.5 mg	1,86	36.71	-
12	Jakarta Selatan	BD3	244.3 mg	1,89	31.97	-
13	Jakarta Selatan	BD4	242.9 mg	1,92	36.98	-
14	Jakarta Selatan	P1	272.2 mg	1,86	32.29	-
15	Jakarta Selatan	P2	274.9 mg	1,8	30.85	-
16	Jakarta Selatan	P3	279.4 mg	1,89	37.08	-
17	Jakarta Selatan	BM1	264.8 mg	1,84	-	-
18	Jakarta Selatan	BM2	283.9 mg	1,82	-	-
19	Jakarta Selatan	BM3	252.4 mg	1,82	30.06	-
20	Jakarta Pusat	JB1	268.4 mg	1,85	32.41	-
21	Jakarta Pusat	JB2	253.1 mg	1,88	30.66	-
22	Jakarta Pusat	JB3	274.8 mg	1,87	27.54	-
23	Jakarta Pusat	G1	249.6 mg	1,87	30.82	-
24	Jakarta Pusat	G2	243.4 mg	1,89	27.87	-
25	Jakarta Pusat	G3	263.3 mg	1,84	28.77	-
26	Jakarta Pusat	BR1	297.8 mg	1,85	28.17	-
27	Jakarta Pusat	BR2	264.5 mg	1,88	27.38	-
28	Jakarta Pusat	BR3	294.3 mg	1,83	27.22	-
29	Jakarta Timur	PB1	264.1 mg	1,8	34.86	-
30	Jakarta Timur	PB2	241.2 mg	1,85	33.99	-
31	Jakarta Timur	PB3	233.3 mg	1,87	-	-
32	Jakarta Timur	D1	218.9 mg	1,85	31.85	-
33	Jakarta Timur	D2	272.6 mg	1,82	27.02	-
34	Jakarta Timur	D3	248.7 mg	1,82	27.96	-
35	Jakarta Timur	S1	257.7 mg	1,87	37.39	-
36	Jakarta Timur	S2	252.2 mg	1,86	30.94	-
37	Jakarta Timur	S3	273.8 mg	1,81	29.84	-
38	Jakarta Barat	M1	263.8 mg	1,85	30.89	-
39	Jakarta Barat	M2	284.7 mg	1,86	36.59	-
40	Jakarta Barat	M3	283.5 mg	1,87	30.63	-
41	Jakarta Barat	JG1	262.6 mg	1,86	31.91	-
42	Jakarta Barat	JG2	277.7 mg	1,85	31.33	-
43	Jakarta Barat	JG3	247.3 mg	1,82	29.59	-
44	Jakarta Barat	JK1	271.8 mg	1,88	30.69	-

45	Jakarta Barat	JK2	267.5 mg	1,85	32.48	-
46	Jakarta Barat	JK3	262.2 mg	1,87	27.41	-
47	Negative cont. (C-)				-	-
48	Positive cont. (C+)				16.26	+

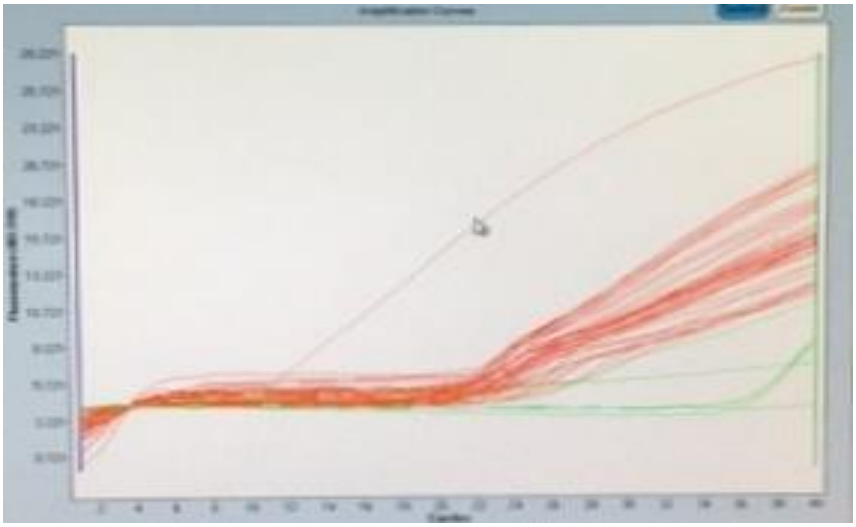

Figure 1. The Result of Real Time–PCR Quantinova Sybr Green of Rat

The results obtained in forty-one samples that were indicated positive but had Cp values that were far different from positive controls could be due to different DNA concentrations in each sample that had not been normalized so it has different Cp values. A significantly different Cp value can also be caused by amplification of bovine DNA because the Cp produced is in the range 27.02 - 36.98, while the specific Cp in rat DNA is in the range of 16-19. According Raharjo (2019), who was doing RT – PCR using ND-1 primers to amplify 294 bp, much longer than common RT – PCR primer which usually targeted less than 100 bp found that Cp / Cq value of mice DNA was range between 17.99 to 19.00 with an average of 18.45.

CONCLUSION

From all of the samples was tested showed that forty-one samples were amplified but with huge different Cp values with positive controls. So that even though all of the samples were amplified, they did not indicate that the samples were positive, the amplification curve that occurred in some samples showed that the DNA in the sample was amplified with Sybr Green but not the Rat DNA. In some samples, there was no amplification curve which could be caused by the low concentration of DNA in the sample and make Sybr Green was not entirely amplified with the sample of DNA.

DAFTAR PUSTAKA

Ali, M.E., Hashim, U., Mustafa, S., Man, Y.B.C., Dhahi, Th.S., Kashif, M., Uddin, M.K., Hamid, S.B.A. 2012. Analysis of Pork Adulteration in Commercial Meatballs Targeting Porcine – Specific Mitochondrial Cytochrome B Gene by Taqman Probe Real – Time Polymerase Chain Reaction. Journal of Meat Science 91; 454 – 459.

Aziz, A.Y. and Vui, C.N. 2012. The Role of Halal Awareness and Halal Certification in Influencing Non-Muslim's Purchase Intention. University Putra Malaysia. Third International Conference on Business and Economic Research (3rd ICBER 2012) Proceeding, ISBN: 978-967-5705-05-2.

Bohari, A. M., Hin, C. W. and Fuad, N. 2013. The Competitiveness of Halal Food Industry in Malaysia : A SWOT ICT Analysis. Geografia Online Malaysia Journal of Society and Space 9 issue 1 (1 - 9) 1, ISSN 2180-2491.

Heinz, G., Hautzinger, P. 2007. Meat Processing Technology For Small To Medium Scale

Producers. FAO Regional Office For Asia and The Pacific, RAP Publication 2007 / 20.

Farag, M.R., Alagawany, M., El-Hack, M. E. A., Tiwari, R., Dhama, K. 2015. Identification of Different Animal Species in Meat and Meat Products: Trends and Advances. Jour. of Adv. Anim. Vet. Sci. 3(6): 334 – 346.

Ilhak, O.I., Arslan, A. 2007. Identification of Meat Species by Polymerase Chain Reaction (PCR) Technique. Turk. J. Vet. Anim. Sci: 31(3): 159 – 163.

Janosi, A. 2006. Species Specific Detection of Meat by Polymerase Chain Reaction Techniques. Corvinus University of Budapest, Budapest, Rumania.

Joshi, M., Deshpande, J.D. 2010. Polymerase Chain Reaction: Methods, Principles and Application. International Journal of Biomedical Research 1 (5) 81 – 97.

Jonker, K.M., Tilburg, J.J.H.C., Hagele, G.H., Boer, E.D. 2008. Species Identification in Meat Products Using Real – Time PCR. Food Aditives and Contaminants: Part A, 25:5, 527 – 533.

Mursyidi, A. 2012. The Role Of Chemical Analysis in the Halal Authentication of Food and Pharmaceutical Products. J. Food Phar.Sci. 1 (2013) 1 – 4.

Murugaiah, C., Noor, Z.M., Mastakim, M., Bilung, L.M., Selamat, J., Radu, S. 2009. Meat Species Identification and Halal Authentication Analysis Using mitochondrial DNA. Elsevier Journal of Meat Science 83 (2009) 57 – 61.

Raharjo, T.J., Pratama, G.A., Nuryanti, I., Pratiwi, R. 2019. Forgery Detection Beef With Mice Meat (Mus muculus in Meatballs Using Real – Time Polymerase Chain Reaction (Real – Time PCR) Primer Specific for a Target Mitochondrial DNA ND-1Gene. Indones. J. Chem., 2019 (1), 89 – 96.

Sahilah, A.M., Norhayati, Y., Norrakiah, A. S., Aminah, A. and Wan Aida, W. M. 2011. Halal Authentication of Raw Meats Using PCR Amplification of Mitochondrial DNA. International Food Research Journal 18(4): 1489-1491 (2011)

Salehudin, I. 2010. Halal Literacy: A Concept Exploration and Measurement Validation. Dept. of Management, Faculty of Economics, University of Indonesia. Asean Marketing Journal, June 2010 – Vol. II – No. 1.

Unajak, S., Meesawat, P., Anyamaneeratch, K. Anuwareepong, D., Srikulnath, K., Choowongkomon, K. 2011. Identification of Species (Meat and Blood Samples) Using Nested-PCR Analysis of Mitochondrial DNA. African Journal of Biotechnology Vol. 10(29), pp. 5670-5676, 22 June, 2011.

Widyasari, Y. I., Sudjadi., Rohman, A. 2015. Detection of Rat Meat Adulteration in Meat Ball Formulations Employing Real Time PCR. Asian Journal of Animal Sciences 9 (6): 460 – 465, 2015.

Zidek, R., Bajzik, P., Marsalkova, L., Golian, J. Detection of Counterfeit Beef Using Real – Time PCR. 2011. Dept. of Hygiene and Food Safety, Slovak University of Agriculture in Nitra.